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Protective Effects of *Curculigo latifolia* Root Extract on Testicular Function in Mice Exposed to 7,12-Dimethylbenz(A)Anthracene

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Abstract. The testis, a vital reproductive organ in males, is highly susceptible to damage from chemotherapy. The root of *Curculigo latifolia* contains secondary metabolites that have potential as anticancer agents and protective agents for reproductive health. This study aims to evaluate the protective potential of crude extract from *C. latifolia* in suppressing testicular carcinogenesis induced by DMBA in vivo. This study employed a completely randomized design with five treatment groups, including negative and positive controls, as well as three treatment groups receiving doses of *C. latifolia* root extract at 300, 600, and 900 mg/Kg body weight. Testicular carcinogenesis was induced by oral administration of DMBA at a dose of 20 mg/Kg body weight twice a week for six weeks. The data of sperm quality, including motility, viability, sperm concentration, and testicular microanatomical structures, analysed by ANOVA one-way test, revealed significant effects from oral administration of *C. latifolia* root extract ($p < 0.05$). Overall, *C. latifolia* root extract improves sperm quality and protects the testicular microanatomical structure against DMBA exposure. Novelty in this study explores the local herbal agent potential (*C. latifolia* root extract), decreasing reproductive damage and alopecia from DMBA carcinogenic effects. Thus, these findings directly contribute to reaching Sustainable Development Goal 3 (SDG3) by ensuring healthy lives and promoting well-being in all aspects for all ages.

Keywords: 7,12-dimethylbenz[a]anthracene (DMBA); *Curculigo latifolia* root extract; BALB/c mice; sperm quality; testicular microanatomy

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INTRODUCTION

Cancer is a disease characterized by the loss of sensitivity to internal regulatory mechanisms, leading to the continuous proliferation of abnormal cells (Katz et al., 2016; Kerdelhu et al., 2016). Cancer is one of the leading causes of death worldwide, with approximately 9.1 million deaths reported annually (Brown et al., 2023). Among cancer types observed in Indonesia, the testicular cancer is one of 28th common cancer types suffered by adult men (Ferlay et al., 2024).

7,12-dimethylbenz(a)anthracene (DMBA) is a chemical agent commonly used to induce cancer in experimental animals, as it transforms normal tissues into abnormal tissues through increased production of free radicals within the cells (Fishbein et al., 2020). Elevated levels of free radicals disrupt the cell cycle and DNA repair pathways, leading to DNA lesions (Hwa et al., 2020). Oral administration of DMBA at a dose of 20 mg/kg body weight, twice a week for six weeks, has effectively induced cancer cell formation in mice, as indicated by increased leukocyte counts and abnormalities in hematopoietic cells (Nurhayati, 2024).

Cancer therapy commonly involves surgical intervention, radiotherapy, and or chemotherapy. Among these modalities, chemotherapy is most frequently used due to its cytotoxic effects on cancer cells. However, this treatment is associated with various side effects, including gonadotoxicity, impaired spermatogenesis, and temporary or permanent infertility (Sharma, 2017). Gonadal cells are highly vulnerable to cytotoxic agents' exposure, since proliferating

germ cells are exposed to such agents, sperm quality declines, followed by disruption in reproductive performance (Ulviena & Hadi, 2023).

Traditional Chinese Medicine (TCM) is widely recognized as a complementary treatment due to its potential to inhibit cancer metastasis and progression (Huang et al., 2018). In recent years, research on the bioprospecting of herbal plants in Indonesia has grown progressively (Pratiwi & Nurlaeni, 2020). One of the undiscovered herbal potentials is from *Curculigo orchioides* extract, in which the previous study from (Singh & Gupta, 2008) found that a concentration of 10 µg/mL of *C. orchioides* extract obviously suppressed the progression and metastasis of human breast cancer cells (MCF-7 cell line).

C. orchioides contains various bioactive compounds, including polysaccharides, saponins, flavonoids, glycosides, and phenolic substances. Curculigoside is a distinctive and dominant phenolic derivative found in this species (Chen et al., 2017; Wang et al., 2021). The antioxidant and anticancer properties of *C. orchioides* extract have been shown to suppress oxidative stress pathways, resulting in significant cytotoxic effects against several cancer cell lines, including HeLa, MCF-7, and HepG2 (Hejazi et al., 2018). However, the study of another species from the *Curculigo* genera such as *Curculigo latifolia* is still rare, while this species is assumed to have a similar potential as a cancer inhibitor due to its close relationship to *C. orchioides* (Muthiam et al., 2025). Therefore, the study is crucial to explore the protective effects of *C. latifolia* extract against DMBA-induced testicular toxicity in vivo, which is still unobserved, mainly on sperm quality and testicular anatomical structure.

This study employed male mice induced with 7,12-dimethylbenz[a]anthracene (DMBA) to produce carcinogenic effects on the testicular organ, followed by oral administration of *C. latifolia* root extract at various doses. This research is expected to provide information on the preventive effects against carcinogenic exposure and to demonstrate the therapeutic potential in addressing disorders of the male reproductive system. The significance of this study lies in providing scientific evidence for the potential of *C. latifolia* as a natural chemoprotective or chemopreventive agent. Furthermore, this research optimizes the utilization of natural candidates in the prevention of testicular cancer and male reproductive disorders scientifically. Therefore, it obviously supports the development of local herbal products aimed at improving quality of life through more affordable treatments while minimizing side effects.

METHODS

Ethical Approval

Ethical approval for this study was granted by the Health Research Ethics Committee of UNISA Bandung, under certificate number 1299/KEP. 01UNISA-BANDUNG/V/2025.

Extraction of *Curculigo latifolia* root using the maceration method

The roots of *C. latifolia* were collected, washed, dried, and ground into a fine powder. A total of 185 g of the powder was macerated in food-grade ethanol (96%) at a ratio of 1:4 (w/v) for 72 hours at room temperature in the dark, with occasional stirring. The resulting filtrate was separated from the solvent using a rotary evaporator at a temperature of 78°C. The crude extract was weighed and subsequently used as the treatment material (Laxmi & Begum, 2022).

Animal model and acclimatization

A total of 25 male BALB/c mice, aged 8–10 weeks and weighing 29.12 ± 0.98 g, were used as our experimental units. Healthy animals with normal activity were selected. The studied animals were obtained from the Institut Teknologi Bandung and acclimatized for seven days at the Biology Laboratory of Universitas Muhammadiyah Bandung.

Induction of DMBA and administration of *C. latifolia* root extract

This study was designed using a Completely Randomized Design (CRD), consisting of five groups with five replicates in each group. Mice were orally induced with 0.1 mL of 7,12-dimethylbenz[a]anthracene (DMBA) at a dose of 20 mg/kg body weight, administered twice a week for six weeks. In addition, 0.1 mL of *C. latifolia* root extract was orally administered daily for six weeks (Minari & Okeke, 2014). Corn oil (DMBA solvent) + mineral water (C-), DMBA + mineral water (C+), DMBA + extract 300 mg/Kg (D1), DMBA + extract 600 mg/Kg (D2), and DMBA + extract 900 mg/Kg body weight (D3). The mice were provided with 5 g/day of standard feed (Hy-Provit) and had access to mineral water ad libitum. The animals were maintained at room temperature under a controlled light cycle of 8 hours light and 16 hours dark.

Spermatozoa Preparation

The mice were euthanized in the sixth week (day 43). Dissection was performed in the scrotal region to collect the cauda epididymis, which was then weighed and homogenized in physiological saline solution (NaCl) at a ratio of 1:9 (w/v). Chloroform was used as the euthanasia agent (Martinez, 2022; Vieira, C. P., Procópio, M. S., & Avelar, 2024).

Sperm Motility: Sperm motility analysis was performed using a light microscope at 400× magnification. A total of 10 µL of diluted sperm sample (10x dilution) was placed on a glass slide for observation. One hundred spermatozoa were randomly observed across several different fields of view. Sperm motility was calculated as the percentage of progressively moving spermatozoa out of the 100 observed cells.

Sperm Viability: Sperm viability analysis was performed by preparing 10 µL of diluted sperm sample (10x dilution) mixed with 10 µL of 5% eosin stain. Observations were conducted on 100 cells randomly selected across several different fields of view. Viable spermatozoa were identified as unstained (transparent) cells, whereas cells that absorbed the eosin stain were classified as non-viable. Viability was expressed as the percentage of viable cells out of the 100 observed cells.

Sperm concentration: Sperm concentration was determined using a hemocytometer by observing five medium squares. A total of 10 µL of diluted sperm sample (10x dilution) was placed into the hemocytometer chamber and allowed to settle for one minute to ensure even distribution before observation under a light microscope at 400x magnification. Sperm concentration was calculated using the standard erythrocyte cell counting formula.

Morphology: Sperm morphology was evaluated from smear preparations stained with eosin. Morphological observations were performed under a light microscope at 400× magnification, focusing on the head, neck, and tail regions of the spermatozoa. A total of 50 cells were randomly observed for morphological assessment.

Acrosome-to-cytoplasm ratio: the acrosome-to-cytoplasm ratio of sperm heads was analyzed from smear preparations stained using eosin. The measurement was conducted by comparing the length of the acrosome to the total length of the head, and the results were expressed as a percentage.

Histological Preparation of Testis

Tissue samples were fixed in Neutral Buffered Formalin (NBF), dehydrated through a series of graded ethanol (70%, 80%, 90%, and 100%), cleared in xylene, infiltrated with a graded xylene-paraffin mixture, and embedded in pure paraffin at a melting point of 50–60 °C. Paraffin blocks were sectioned to a thickness of 5 µm using a rotary microtome, and the testicular tissue sections were adhered to glass slides coated with 1% gelatin. The well-adhered sections were stained with Mayer's Hematoxylin and Eosin (Hillary et al., 2022; Wijayanti et al., 2017).

Histomorphometry: histomorphometry evaluation includes the diameter of seminiferous tubules, the diameter of seminiferous lumen, the area of seminiferous tubules, and the area of seminiferous lumen. Measurement of histomorphometry parameters was conducted on 20 randomly selected seminiferous tubules. The diameter of the seminiferous tubules and lumen was obtained from the average of the long and short diameters (Figure 2.1). The area of the tubules and lumen was calculated using the formula for the area of an ellipse (Saoza et al., 2021). Histomorphometry measurements were performed using the following formula:

- Diameter of seminiferous tubules= $\frac{ST1+ST2}{2}$
- Diameter of lumen=$\frac{SL1+SL2}{2}$
- Area of seminiferous tubules= π x $\frac{1}{2}$ST1 x $\frac{1}{2}$ST2
- Area of seminiferous lumen = π x $\frac{1}{2}$SL1 x $\frac{1}{2}$SL2
- Proportion of spermatogenic cells in tubules $=\frac{tubulus\ area-lumen\ area}{tubulus\ area}\ x\ 100\%$

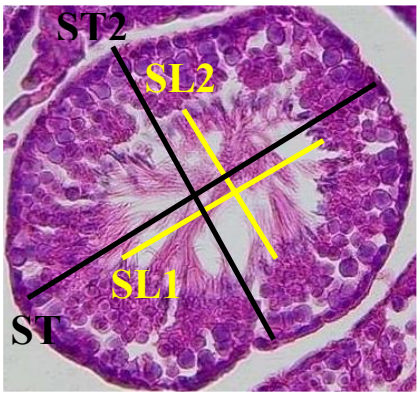

Figure 1. Measurement of seminiferous tubules and seminiferous lumen. ST: seminiferous tubules (SL1: long diameter; SL2: short diameter); SL: seminiferous lumen (SL1: long diameter; SL2: short diameter)

Histopathology of the Testis: the assessment of the histopathological condition of the testis is based on a grading system consisting of eight levels: (1) intact seminiferous tubules, with little or no vacuolation; (2) vacuoles present in the basal germinal area; (3) vacuoles present in the apical germinal area; (4) vacuoles present in both the basal and apical germinal areas; (5) degeneration of germ cells within the seminiferous lumen; (6) seminiferous tubules containing only basal germinal cells; (7) seminiferous tubules containing only Sertoli cells; and (8) seminiferous tubules exhibiting complete degeneration, with no Sertoli or germ cells present. Criteria 1 are classified as normal seminiferous tubules, criteria 2 and 3 as mild damage, criteria 4 and 5 as moderate damage, and criteria 6-8 as severe damage. Histopathological observations of the testis were conducted on 40 randomly selected seminiferous tubules in each mice (Souza et al., 2021).

Data Analysis

Sperm morphology and testicular microanatomy data were described and presented in the form of micrographs. Parameters described as percentage data, while sperm concentration data were log-transformed to improve normal distribution in the Shapiro-Wilk test. Then, homogeneity was checked by Levene's test. After that, one-way ANOVA was used to analyze quantitative data, including sperm quality, sperm cell cytometry, histomorphometry, and histopathology, followed by Tukey's HSD post hoc test at a $p < 0.05$ level if significant results occur (Lavanya et al., 2021).

RESULTS AND DISCUSSION

Body weight, testis weight, and alopecia in male BALB/C mice

Body weight and testis weight post-treatment were relatively similar across all treatment groups ($p > 0.05$). Weight gain was observed only in the C- treatment group. All mice exhibited alopecia in the C+ treatment group, while the incidence decreased in the groups receiving *C. latifolia* extract (Table 1). Mice in the C− group exhibited normal body morphology with dense and evenly distributed fur, without any signs of alopecia (Figure 2A). Conversely, mice in the C+ group showed localized alopecia in the head and cervical regions, causing the exposed skin in these areas to appear purplish (Figure 2B). Testis from C− mice appeared normal, with a smooth surface, firm consistency, absence of lesions, and a pale yellow coloration (Figure 2C). In the C+ group, two of five mice showed black spots on the testicular surface (indicated by yellow arrows in Figure 2D). These findings indicate testicular damage in mice exposed to DMBA for six weeks, while the administration of *C. latifolia* root extract attenuated the adverse effects of DMBA.

Exposure to DMBA disrupts the architecture of melanocytes within the epidermis and hair follicles, characterized by shortened and aggregated dendrites, thereby impairing normal cellular organization. After six weeks of DMBA induction, 100% of mice in the C+ group developed alopecia. This finding is aligned with previous findings (Kaur et al., 2024), which reported that DMBA induces hair loss through follicular degeneration. DMBA causes damage to hair root sheaths and follicles, leading to hair loss. DMBA has also been reported to cause testicular damage due to the accumulation of necrotic cells, increased oxidative stress, and localized hemorrhage.

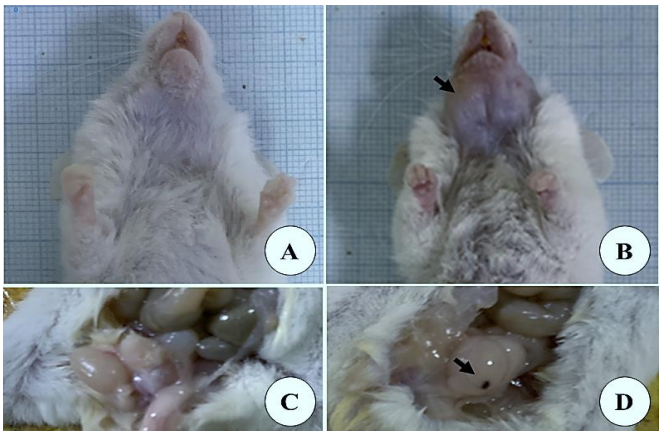

Figure 2. Hair follicles and the morphology of the testis

Table 1. Body weight, testis weight, and alopecia in mice during the study

Treatment	C-	C+	D1	D2	D3
Initial body weight (g)	29.63±1.8[a]	32.87±2.38[a]	33.95±0.94[a]	32.19±3.01[a]	30.81±1.93[a]
Final body weight (g)	30.66±1.53[a]	31.64±3.31[a]	32.86±4.56[a]	31.06±2.90[a]	29.71±6.25[a]
Weight gain (%)	+1.03	-1.23	-1.09	-1.13	-1.10
Left testis (%)	0.09±0.05[a]	0.09±0.04[a]	0.10±0.03[a]	0.10±0.03[a]	0.07±0.03[a]
Relative left testicular weight (%)	0.28±0.17[a]	0.28±0.11[a]	0.30±0.06[a]	0.31±0.11[a]	0.25±0.10[a]
Right testis (%)	0.10±0.04[a]	0.08±0.02[a]	0.10±0.01[a]	0.11±0.03[a]	0.09±0.02[a]
Relative right testicular weight (%)	0.33±0.13[a]	0.24±0.08[a]	0.29±0.03[a]	0.35±0.12[a]	0.32±0.14[a]
Alopecia (%)	0	100	40	40	20

Note: Superscript letters in the same row indicate no significant difference in the Tukey HSD post hoc test at 5%

Table 1. Sperm quality profile based on motility, viability, and sperm cell concentration in BALB/c mice

Treatment	C-	C+	D1	D2	D3
Sperm motility (%)					
Progressive	42.86±2.64[d]	13.80±3.22[a]	26.45±1.54[b]	32.37±2.02[c]	33.73±0.59[c]
Non progressive	12.94±4.46[b]	10.13±3.22[a]	11.14±2.51[a]	10.64±3.13[a]	9.10±2.98[a]
Immotile	44.27±4.26[a]	76.07±4.89[c]	62.40±1.74[b]	56.98±4.27[b]	57.17±2.65[b]
Viability (%)	62.34±2.39[b]	28.54±3.87[a]	59.35±2.95[b]	59.14±1.37[b]	60.09±0.68[b]
Sperm concentration (10^6 cell/mL)	167.7 ± 64.0[c]	79.5 ± 10.8[a]	91.0 ± 10.3[ab]	117.0 ± 10.3[bc]	145.3 ± 50.4[c]

Note: Superscript letters in the same row indicate no significant difference in the Tukey HSD post hoc test at 5%

Profile of sperm motility, viability, and concentration in male BALB/c mice

Oral administration of *C. latifolia* root extract showed significant differences among treatment groups in progressive motility, viability, and sperm concentration (cells/mL). The administration of *C. latifolia* extract increased progressive motility, viability, and sperm concentration compared to the positive control group (C+) ($p < 0.05$). The D3 treatment group demonstrated results that were comparable to those of the negative control group (C−) (Table 1). These findings indicate the protective role of *C. latifolia* in preventing sperm quality decline caused by the adverse effects of DMBA

Mice treated with *C. latifolia* root extract expressed increases of progressive motility, viability, and sperm concentration (cells/mL). The D3 treatment group (900 mg/kg body weight) demonstrated the most favorable outcome, with results approaching those of the negative control group (C−). These findings indicate that *C. latifolia* can counteract the adverse effects of DMBA exposure and has potential as a natural therapeutic source for preventing male infertility caused by toxic exposure to the reproductive system. This result is consistent with a previous study from (Jaafar et al., 2017) found the improvements in sperm quality, particularly in motility and sperm count, following administration of *C. latifolia* root extract at a dose of 500 mg/kg body weight.

DMBA has been negatively reported to affect sperm quality by several mechanisms, including the induction of DNA damage, increased oxidative stress, enhanced lipid peroxidation, reduced ATP production, mitochondrial dysfunction, and the triggering of primary abnormalities in spermatogenesis (Jaafar et al., 2017). In contrast, *C. latifolia* exhibits protective properties against oxidative stress and possesses antioxidant and anti-inflammatory activities associated with its bioactive metabolites such as flavonoids, polyphenols, and saponins (Karimani et al., 2021; Morales et al., 2021). The bioactive compounds such as curculigoside and phlorizin in *C. latifolia* are responsible for suppressing cellular damage-induced proteins, maintaining energy production, and preserving mitochondrial integrity, thereby enhancing sperm viability and motility (Barbagallo et al., 2020; Bravo et al., 2024; Taufik et al., 2024). Therefore, *C. latifolia* has strong potential as a promising protective agent for maintaining sperm quality against toxic exposure such as DMBA.

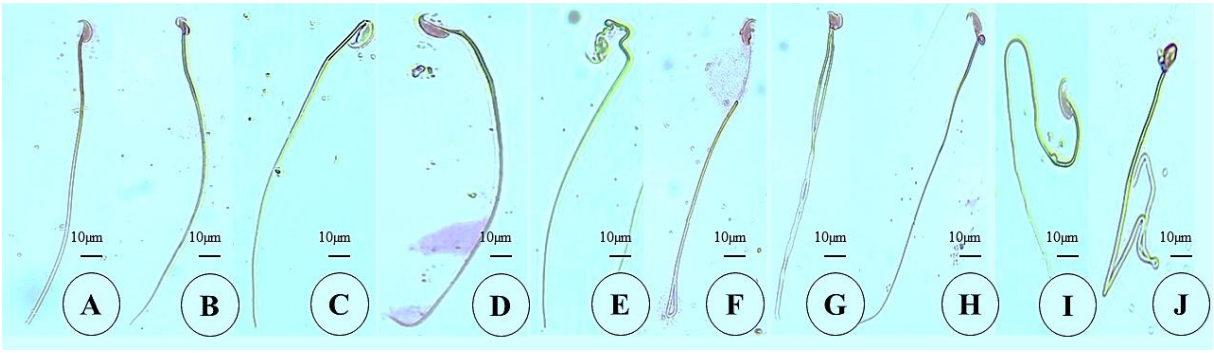

Figure 3. Characteristics of normal and abnormal spermatozoa morphology

Structure and cytometry of sperm cells in male BALB/c mice

Normal sperm cell structure is characterized by intact head, neck, and tail regions. The head is falciform or hook-shaped, the neck is straight and relatively short, and the tail is straight and relatively long (Figure 3A). Abnormal sperm structures include a head that is not falciform and excessively thin (Figure 3B), a tilted head (Figure 3C), a bent neck (Figure 3D), a wavy neck (Figure 3E), the presence of a neck droplet (Figure 2F), a coiled neck (Figure 2G), an uneven neck (Figure 2H), a coiled tail (Figure 2I), and a double tail (Figure 2J).

Normal spermatozoa are characterized by a falciform or hook-shaped head. The head contains a nucleus that carries genetic material (DNA) and an acrosome that contains specific enzymes essential for penetrating the oocyte membrane during fertilization. The neck region is composed of mitochondria where ATP is produced to supply the energy required for sperm motility. The tail functions as the locomotor apparatus and is divided into three parts: the connecting piece, principal piece, and end piece. Abnormalities in any part of the sperm cell can lead to impaired sperm motility and reduced fertilization capability, potentially resulting in infertility. Primary sperm abnormalities originate from disturbances during spermatogenesis, whereas secondary abnormalities arise from defects during epididymis maturation or post-ejaculation. Common sperm abnormalities include double heads, excessively thin heads, absence of the acrosome, bent heads, broken tails, and coiled tails (Ray et al., 2017).

The ANOVA analysis showed significant differences in head size, neck length, tail length, and acrosome-to-cytoplasm ratio among the experimental groups ($p < 0.05$). The smallest head and neck dimensions were observed in the D3 group (9.10 µm and 23.06 µm, respectively), whereas the largest head size was recorded in the D2 group (10.04 µm). The shortest tail length was found in the C+ group (52.5 µm), while the longest tail was observed in the D3 group (74.62 µm). However, the acrosome ratio among treatment groups remained within the physiological range (40–70%). Structural abnormalities in sperm caused by DMBA exposure may disrupt spermatogenesis and ultimately contribute to infertility (Table 2).

DMBA leads to ROS disrupting testicular germ cells and testosterone levels. An increase in ROS levels in spermatogenic cells can lead to DNA fragmentation and cause spermatogenic cell abnormalities (Bisht et al., 2017). The flavonoid and phenolic content of *C. latifolia* root extract neutralizes ROS levels by increasing endogenous antioxidant enzymes SOD, catalase, and GPx, thus protecting the membrane and mitochondrial structure of sperm cells. Furthermore, antioxidants play a role in reducing germ cell damage by preventing DNA fragmentation and maintaining spermatogenic cell integrity (Durairajanayagam, 2019).

The more frequent DMBA exposure, the higher risk of morphological abnormalities of sperm cells, particularly in the head region, including irregular shape, acrosome loss, and reduced size. Specifically, sperm head damage caused by oxidative stress can induce DNA damage, resulting in decreased testosterone levels and disruption of spermatogenesis. Besides, impaired chromatin condensation during the cell cycle contributes to alterations in cell size and head structure. Sperm head fragmentation directly reduces sperm viability. Furthermore, DMBA exposure elevates the production of reactive oxygen species (ROS), resulting in mitochondrial damage in the sperm neck region. Structural damage to the neck and tail ultimately impairs sperm motility due to reduced ATP availability (Kaur et al., 2024; Yogo, 2022).

Table 2. Abnormalities, cytometry, and acrosome ratio of spermatozoa in BALB/c mice

Treatment	C-	C+	D1	D2	D3
Proportion of morphologically normal spermatozoa (%)	92.5 ± 5[b]	71.5± 2.85[a]	86 ± 2.85[b]	89 ± 6.27[b]	91.5± 2.85[b]
Cytometry					
Head (µm) ± SD	10.24 ± 0.08[b]	9.97 ± 0.13[a]	10.05 ± 0.14[b]	10.10 ± 0.11[b]	10.15 ± 0.10[b]
Neck (µm) ± SD	46.09 ± 3.92[c]	26.14 ± 2.12[a]	30.56 ± 1.07[b]	42.50 ± 8.29[bc]	45.19 ± 1.69[bc]
Tail (µm) ± SD	71.80 ± 1.31[d]	52.50 ± 2.24[a]	65.90 ± 1.24[b]	68.38 ± 0.53[c]	70.65 ± 0.95[d]
Acrosome ratio (%) ± SD	65.80 ± 0.04[a]	70.80 ± 0.02[a]	69.00 ± 0.03[a]	65.00 ± 0.01[a]	68.10 ± 0.07[a]

Note: Superscript letters in the same row indicate no significant difference in the Tukey HSD post hoc test at $p< 0.05$

Table 3. Testicular histomorphometry of male BALB/c mice

Treatment	C-	C+	D1	D2	D3
Size of tubules (µm)	181.15±9.98[a]	176.93±6.71[a]	174.81±8.77[a]	174.95±16.72[a]	176.05±14.24[a]
Size of lumen (µm)	60.06±9.98[a]	91.62±9.36[c]	77.09±4.38[bc]	68.90±9.94[ab]	70.20±8.01[ab]
Tubules area (μm^2)	26481.19± 4482.99[a]	24582.59± 2669.32[a]	22014.29±5364.16[a]	23196.06± 4143.74[a]	23810.31 ±3761.89[a]
Lumen area (μm^2)	3210.59 ±73.80[a]	6750.60 ±1537.31[b]	4403.21 ±577.28[a]	3665.51± 1163.54[a]	3733.42 ±857.98[a]
Tubule area: lumen area	16.53:1[b]	4.26:1[a]	7.56:1[a]	8.24:1[a]	7.63:1[a]
Proportion spermatogenic in tubules (%)	88.29 ±3.34[c]	72.89 ±4.44[a]	78.21 ±4.72[ab]	84.92 ±2.36[c]	84.53 ±1.39[bc]

Note: Superscript letters in the same row indicate no significant difference in the Tukey HSD post hoc test at $p< 0.05$

The size of morphological sperm structures strongly affects successful fertilization. Since genetic material is contained within the sperm head, this specific part plays an important role in attaching ovarium. This current study found that sperm head size was roughly 10 µm, which was smaller than the 9 µm sperm head size in the positive control. Possibly, telomer reduction or aging is responsible for decreasing sperm head as observed by (Rocca et al., 2016) that telomer actively stabilizes genome structures during cell division. Consequently, telomer reduction hampers spermatogenesis, leading to abnormalities of morphological spermatozoa, including the small sperm head phenomenon. This phenomenon often declines sperm's ability to reach the pellucid zone and attach in ovarium membranes properly due to unstable, fragmented DNA.

The size of the neck and tail spermatozoa contributes significantly to successful fertilization. Mitochondria within the neck of a sperm cell play a crucial role in sperm motility, succeeding fertilization. In detail, the shorter the neck size, the smaller the mitochondria it contains, which obviously decreases sperm motility compared to the longer neck size. Additionally, sperm tail size also influences sperm motility, in which shorter tails result in less motile sperm (Castellini et al., 2024).

Histomorphometry of the testis in male BALB/c mice

The one-way ANOVA exhibited significant differences in testicular histometry among groups. DMBA exposure increased the diameter and area of the seminiferous tubule lumen, while decreasing the ratio of seminiferous tubule area to lumen area and reducing the proportion of spermatogenic cells within the seminiferous tubules. The proportions observed in the D1, D2, and D3 treatment groups approached those of the negative control (C−) but differed significantly from the positive control (C+) ($p < 0.05$; Table 3). These findings indicate that *C. latifolia* provides a protective effect.

A reduction in the number of spermatogenic cells within the seminiferous tubules indicates an impairment of the spermatogenesis process. Administration of DMBA at a dose of 10 µg/100 g BW (approximately equivalent to 10 mg/kg BW) has been reported to decrease the population of spermatogenic cells in the seminiferous tubules of rats (Kalucka et al., 2015; Yildirim et al., 2018; Aref et al., 2023). The root extract of *C. latifolia* is known to contain bioactive compounds such as

flavonoids, alkaloids, and phenolic substances (Haryanto et al., 2023). These compounds have been associated with antioxidant activity through the reduction of free radicals and modulation of apoptotic pathways within the cell cycle (Anchimowicz et al., 2025; Ragusa, 2023). In the *Allium cepa* assay, the root extract of *C. latifolia* at a concentration of 400 µg/mL was shown to reduce the mitotic index by up to 32.7%, indicating its ability to regulate cell division while exhibiting low cytotoxicity toward normal cells (Susanti et al., 2025). These findings are consistent with previous studies reporting protective effects on histomorphometric parameters using *Lagenaria siceraria* extract administered orally at 10 µg/100 g BW in rats exposed to DMBA (Aref et al., 2023).

Histopathology of the testis in male BALB/c mice

In cross-sectional slices, the testis is composed of compact, orderly seminiferous tubules of uniform size (Figure 4A). The seminiferous tubules are round to oval in shape and contain various types of developing spermatogenic cells. Spermatogonia (SG) are germ cells, characterized by their round shape, centrally located nucleus, and location in the basal membrane (MB). Primary and secondary spermatocytes (ST) are difficult to be distinguished, this cell round shape, an oval nucleus, and are located above the spermatogonia. Spermatids (SD) are cells located between the spermatocytes and spermatozoa. Those cells form an oval shape and an oval nucleus. Spermatozoa (SZ) are falciform head and a strand-like tail structure. The size of spermatogenic cells decreases from the basal membrane to the lumen of tubules (L) (Figure 4B). Spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa are arranged closely and sequentially from the basal part of the seminiferous tubule to the seminiferous lumen. Normal seminiferous tubules are characterized by a distinct shape, containing various stages of spermatogenic cells and very few vacuoles (Figure 4C). Mild damage is indicated by the presence of vacuoles in the basal region of the seminiferous tubule, leading to disintegration between the germinal epithelial cells and some spermatogonia (Figure 4D). Another form of mild damage is characterized by vacuoles in the apical region of the seminiferous tubule, resulting in a loose arrangement of spermatogonia (Figure 4E). Moderate damage is marked by vacuoles extending from the basal to the apical regions of the seminiferous tubule (Figure 4F). Another type of moderate damage is indicated by the disintegration of spermatogenic cells in the seminiferous lumen (Figure 4G). Severe damage was characterized by seminiferous tubules composed only of basal cells (Figure 4H). Nuclear damage in spermatogonia is shown as dark, condensed nuclei, commonly referred to as pyknotic nuclei (Figure 4I). Cytoplasmic alterations in spermatogonia, indicated by a reddish appearance consistent with plasma cell infiltration, were also observed (Figure 4J).

Our results revealed significant differences among treatment groups in the proportion of normal seminiferous tubules, and histopathological damage ranged from mild to severe ($p < 0.05$). The C+ treatment exhibited the lowest number of normal seminiferous tubules, while the normal seminiferous tubules in the D2 and D3 groups were relatively similar to those in the C- group. In cases of mild, moderate, and severe damage, the D3 treatment approached and exhibited values relatively comparable to the C- treatment, with significant differences observed when compared to the C+ treatment (Figure J). Based on these findings, the extract of *C. latifolia* may mitigate the carcinogenic effects of DMBA on the histopathological parameters of the testis (Figure 5).

In this study, DMBA had a significant negative effect on both the quality and quantity of sperm. DMBA is a polycyclic aromatic hydrocarbon (PAH) commonly used to induce carcinogenicity in test animals (Hwang et al., 2007). DMBA plays a role in increasing lactate dehydrogenase, which is followed by necrosis; additionally, DMBA also enhances the expression of MDM2 protein, which can inhibit the tumor suppressor p53, increase the expression of cyclin D1 (a protein that drives the cell cycle through the G1 phase), and elevate P27 protein levels, thereby activating the cyclin D/cdk complex that promotes cell division and inhibits apoptosis (Abbaoui et al., 2017). The testis is highly susceptible to the toxic effects of DMBA because the process of spermatogenesis exhibits characteristics similar to the rapid growth of cancer cells (Kaur et al., 2024). A study by Yildirim et al. (2018) on the effects of sodium fluoride (NaF) and DMBA demonstrated that both substances significantly reduced testosterone levels and caused alterations in spermatogenesis, such as an increase in the degree of testicular histopathology.

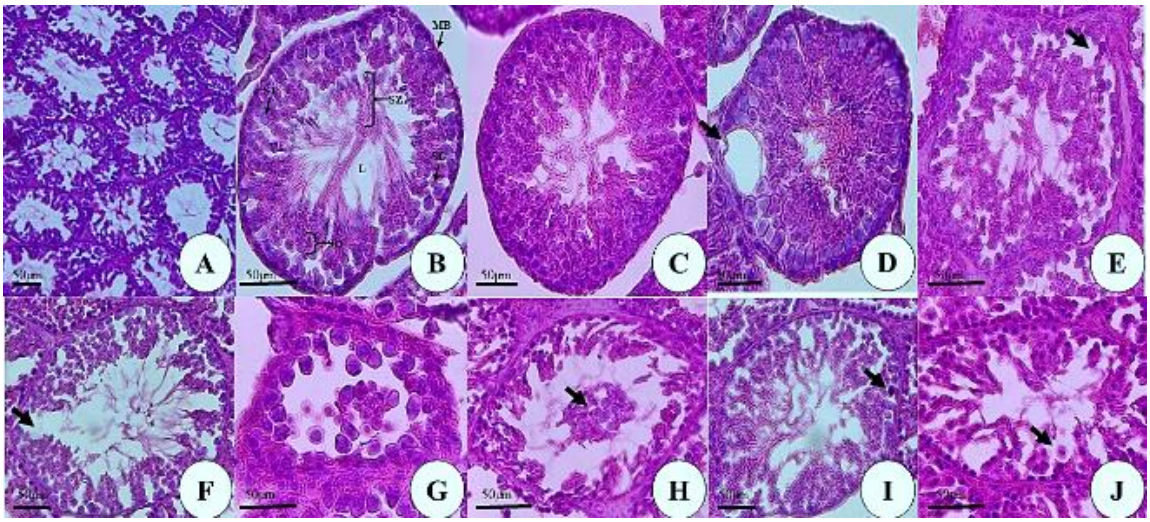

Figure 4. Testicular pathology in male BALB/c mice

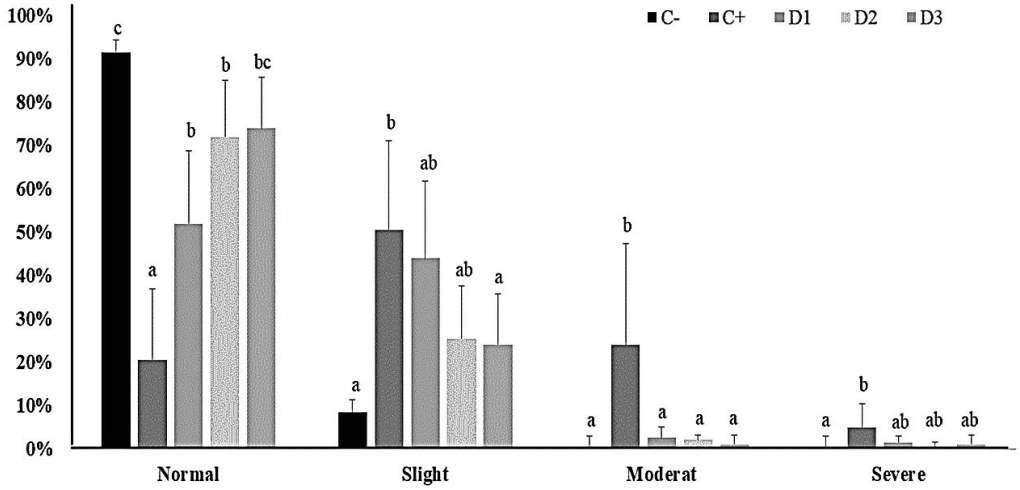

Figure 5. Testicular pathology in male BALB/c mice

C. latifolia has been reported to protect germ cells and improve sperm quality impaired by exposure to carcinogenic compounds (Zabidi et al., 2019). The anticancer potential of norlignane compounds in *C. latifolia* is associated with activation of both intrinsic and extrinsic apoptotic pathways. The intrinsic pathway involves mitochondrial release of cytochrome c followed by caspase-3 activation, whereas the extrinsic pathway is mediated through death receptor (DR5) signaling that triggers caspase-8 and caspase-10 activation in cancer cells (El-Deiry, 2020). Previous study from Zhao et al. (2023) proved that excessive ROS production can be suppressed by Norlignane, as a result of this suppression, DNA damages, down-regulate anti-apoptotic proteins (Bcl-2), up-regulate pro-apoptotic proteins (Bax), and cancer cells induction can be gradually controlled. Moreover, norlignane may inhibit cancer cell proliferation through suppression of the PI3K/AKT/mTOR pathway, leading to decreased VEGF expression, inhibition of angiogenesis, and decreased nutrient supply to tumors (Zhao et al., 2023).

This study demonstrated that oral administration of *C. latifolia* root extract at doses of 300–900 mg/kg BW acted as a protective effect on sperm viability, motility, concentration, cellular integrity, and testicular histology in mice exposed to the carcinogenic agent DMBA. The bioactive compounds within *C. latifolia* root extract contributed to a measurable protective effect on male reproductive parameters. The higher administered doses (600 and 900 mg/kg BW) can be considered as a notable novelty to fill the research gap in this scientific discipline, since the rarity of previous studies has implemented those doses. Additionally, the extract showed potential protective effects against DMBA-induced alopecia.

The benefits of this study offer an alternative protective agent against DMBA-induced

carcinogenic effects on the male reproductive system, particularly in terms of sperm quality and testicular histology. The findings also provide information on an optimal protective dose of *C. latifolia* root extract, identified as 900 mg/kg BW, in mitigating the adverse reproductive effects of DMBA exposure and reducing alopecia. Despite the chemotherapy, the utilization of *C. latifolia* may offer an alternative clinical approach for managing male reproductive disorders and serve as a complementary therapeutic candidate for testicular cancer and a decrease in alopecia degree simultaneously mainly for the general public. This study also aligns with the Sustainable Development Goals (SDGs), particularly Goal 3: Ensure healthy lives and promote well-being for all at all ages. By providing data on the inhibition of DMBA-induced reproductive toxicity, evaluating effective extract dosages, and analyzing histological changes in testicular tissue, this research supports the exploration of local biodiversity as a source of therapeutic agents with potentially fewer side effects.

CONCLUSION

Oral administration of *C. latifolia* root extract improved sperm quality and exerted a protective effect on the testicular structure of DMBA-induced BALB/c mice. Treatment with *C. latifolia* root extract demonstrated protective effects across sperm viability, motility, concentration, cellular integrity, and histological parameters. The extract shows potential as a phytotherapeutic candidate for mitigating testicular toxicity and maintaining male reproductive health. Further studies are warranted to investigate hormonal profiles and gene expression, in order to elucidate the molecular mechanisms by which *C. latifolia* protects against the deleterious effects of the carcinogenic agent DMBA.

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AUTHOR CONTRIBUTION STATEMENT

All authors contributed significantly to the work reported in this manuscript. H conceived and designed the study, conducted the data analysis. CA, SMA, and H was responsible for data collection, H, LHM, AS, CA, SMA and FAP assisted in the literature review, supported data visualization, and provided substantial feedback during the manuscript drafting process. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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